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Effects of drying methods on the antioxidant activities of polysaccharides extracted from *Ganoderma lucidum*

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ABSTRACT

Three polysaccharides (GLP-H, GLP-V and GLP-F) were obtained from *Ganoderma lucidum* by hot air drying, vacuum drying and vacuum freeze drying, respectively. Their chemical compositions were determined, and antioxidant activities were investigated on the basis of superoxide radical, hydroxyl radical, reducing power, DPPH free radical and Ferric-reducing antioxidant power (FRAP) assay. The results showed that three polysaccharides exhibited antioxidant activities in a concentration-dependent manner. Among three polysaccharides, GLP-F and GLP-V had the higher scavenging effects on hydroxyl radicals, superoxide radicals, DPPH free radical, and stronger reducing power than GLP-H. GLP-F showed the stronger antioxidant capacity than GLP-V and GLP-H in FRAP system. GLP-H and GLP-V showed an almost identical pattern in FRAP system ($p > 0.05$). However, GLP-V showed the stronger radical scavenging activities than GLP-H. Available data obtained in in vitro models suggested that vacuum freeze drying was an appropriate and effective treatment for obtaining the polysaccharide from *G. lucidum*.

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1. Introduction

Oxidation is an essential biological process to many living organisms for the production of energy. However, the uncontrolled production of oxygen-derived free radicals is hostile and damaging to cells. It can also cause a chain reaction resulting to the multiplication of new free radicals. The damage they cause includes interference and manipulation of protein, tissue loosening, genetic damage and the promotion of disease and aging. In order to reduce oxidation damage to the human, many synthetic antioxidants are widely used at present. However, recent researches suggested that synthetic antioxidants were restricted due to their potential hazards to health, such as liver damage and carcinogenesis (Grice, 1988; Yuan, Zhang, Fan, & Yang, 2008). Thus, it is essential to develop and utilize effective natural antioxidants to protect the human body from free radicals and reduce risk of many diseases such as heart disease, cancer, arthritis and the aging process (Nandita & Rajini, 2004).

Ganoderma lucidum is a member of the fungus family and is usually used in traditional medicine. The use of *G. lucidum* as a longevity and vigor-promoting "magic herb" dates back more than 2000 years in China. The fruiting bodies, called "Lingzhi", contain a variety of chemical substances. They have been proved to be effective in the treatment of chronic hepatopathy, hypertension, hyperglycemia and neoplasia (Sone, Okuda, & Wada, 1985; Wang et al., 1997). Recent studies show that polysaccharides are one of the main active components of *G. lucidum* and they exhibit many biological activities including anti-tumor (Wasser, 2002), antioxidant (Liu, Wang, Pang, Yao, & Gao, 2010), hypoglycemic, and immune-stimulating effects (Han, Nakamura, & Hattori, 2006; Yang, Wu, & Zhang, 2004).

In the past decades, it has been found that the polysaccharides in plants are not only energy resources but play key biological roles in many life processes as well. Natural polysaccharides have been used in the food industry and in medicine for a long time. The structure and mechanism of bioactive polysaccharides on diseases have been extensively studied, and more natural polysaccharides with different curative effects have been tested and even applied in therapies (Wang & Fang, 2004). Some natural polysaccharides have been demonstrated to play an important role as free radical scavengers in the prevention of oxidative damage in living organism and can be explored as novel potential antioxidants (Ge, Duan, Fang, Zhang, & Wang, 2009; Matkowski, Tasarz, & Szypula, 2008; Yuan et al., 2008). Moreover, previous studies indicated that antioxidant activity of polysaccharides might come from the ability to improve the activity of antioxidant enzymes, scavenge free radicals and inhibit lipid peroxidation (J. Xu et al., 2009). It is generally admitted that the free radicals cause lipid peroxidation, decrease permeation, cause damage of membrane proteins and contribute to cellular inactivation (Borchani et al., 2010).

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Antioxidant activities of polysaccharide can be affected by many factors including its chemical components, molecular mass, structure, conformation, even the drying methods, especially for the components that has been extracted or isolated from the raw material.

Drying has become a widely used way of food processing allowing the extension of the shell-life. However, processing may cause irreversible modifications to the cell wall polysaccharides, affecting their original structure. This may promote important changes in the proposed physiological and pharmacological properties of these polymers (Femenia, 2007). Conventional hot air drying (HD) is the most frequently used and low-cost drying method in food industry. However, significant quality changes of dried products may occur during HD. Vacuum drying (VD) is ideal for materials that would be damaged or changed if exposed to high temperatures. The vacuum removes moisture while preventing the oxidation that can occur when certain materials combine with air. Vacuum freeze drying (FD) is a method of dehydration of frozen materials by sublimation under vacuum and it could produce the high-quality dried foods. However, its major problem is the long drying time needed, which in turn leads to high energy consumption and high capital costs.

Polysaccharides are one of the main biological activities constituents of *G. lucidum*. However, there was no information about the effects of drying methods on antioxidant activities of polysaccharides from *G. lucidum*. The aim of the present study was to investigate the influences of different drying methods (HD, VD and FD) on the antioxidant activity for seeking the potential drying method for the production of active polysaccharides.

2. Materials and methods

2.1. Material

G. lucidum, was obtained from Research Institute of *G. lucidum* (Zhejiang, China). β-Nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ascorbic acid and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, USA). Potassium ferricyanide, ferric chloride and ethanol were purchased from Shanghai Chemical Reagent Company (Shanghai, China). All other chemicals and solvents were analytical grade and used without further purification.

2.2. Polysaccharide extraction from G. lucidum

The fruiting body of *G. lucidum* was pretreated with ethanol to deactivate the endogenous enzymes and remove some soluble materials, and extracted with hot distilled water and precipitated with ethanol to obtain crude *G. lucidum* polysaccharide (GLP) according to our previous method (Li, Fan, & Ding, 2011).

2.3. Drying procedure

Drying experiment of crude GLP was carried out using the three different methods of HD, VD and FD. The HD was done at temperature of 60 °C and air-flow rate of 2.0 ± 0.2 m/s. VD was done at 60 °C and 0.06 MPa of vacuum degree. Crude GLP was frozen at −30 °C and dried in a lab scale vacuum freeze dryer (YT2S-01, Nanjing Yatai Microwave Power Technology Research Institute, China) at 60 °C heating shelf temperature, 100 Pa cavity pressure and −40 °C cold trap temperature. The samples were dried until the moisture contents below the 10%. The polysaccharides obtained from *G. lucidum* by the HD, VD and VF methods were named GLP-H, GLP-V and GLP-F, respectively.

2.4. Analytical methods

Neutral sugar content was examined by phenol–sulfuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as standard reference material. The protein content was determined by using the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as reference material. The uronic acid content was determined by 3-hydroxydiphenyl assay using glucuronic acid as reference material (Blumenkrantz & Asbose-Hansen, 1973).

2.5. Assay for antioxidant activity

2.5.1. Hydroxyl radical scavenging activity

Scavenging effects of GLP on hydroxyl radicals were performed according to the method of Ghiselli et al. with a minor modification (Ghiselli, Nardini, Baldi, & Scaccini, 1998). Briefly, 0.1 mL GLP at the concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL was mixed with 0.8 mL of 200 mmol/L phosphate buffer (pH 7.4), 1.75 mmol/L deoxyribose, 0.1 mmol/L ferrous ammonium sulfate and 0.1 mmol/L EDTA, respectively. The reaction was started by adding 0.1 mL of 1.0 mmol/L ascorbic acid and 0.1 mL of 10 mmol/L H_2O_2. The reaction solution was incubated and reacted with thiobarbituric acid (TBA) and trichloroacetic acid. The absorbance was measured at 532 nm. The percentage of scavenging hydroxyl radical was calculated according to the following equation:

$$\text{scavenging effect}\,(\%) = \left(\frac{1 - A_1}{A_0}\right) \times 100$$

where A_0 is the absorbance of the control (without GLP) and A_1 is with GLP.

2.5.2. Superoxide anion scavenging activity

The scavenging effects of GLP on superoxide radicals were assayed by using the method reported by Li, Zhou, and Han (2006). Briefly, 1 mL NBT solution, 1 mL NADH solution and 0.1 mL of GLP at the concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL were dissolved and mixed, respectively. The reaction was started by adding 1.0 mL of phenazine methosulfate (PMS) solution. The reaction mixture was incubated and measured at 560 nm against a blank sample.

2.5.3. DPPH radical scavenging activity

The DPPH radical scavenging activity test of GLP was carried out according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992) with some modification. Briefly, 1 mL of DPPH solution (0.1 mmol/L DPPH in 95% ethanol) was added with 3 mL GLP at the concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/mL and reacted at room temperature. The mixture was shaken and the absorbance was measured at 517 nm. The percent DPPH radical scavenging effect was calculated according to the following equation:

$$\text{DPPH scavenging effect}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of DPPH solution without GLP and A_1 is with GLP.

2.5.4. Reducing power

The reducing power was determined according to the method of Oyaizu (1986) with some modification. Various concentrations of GLP samples in phosphate buffer (1.25 mL, 0.2 M, pH 6.6) were mixed with potassium ferricyanide (1.25 mL, 1.0%) and were incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added, and the mixture was centrifuged at 650 × *g* for 10 min. The supernatant (2.5 mL) was mixed with ferric chloride (0.25 mL, 0.1%) and measured at 700 nm against a blank.

Table 1
Chemical composition of GLP-F, GLP-V and GLP-H.

Samples	GLP-F	GLP-V	GLP-H
Neutral sugar (%)	55.65 ± 1.53[a]	55.03 ± 2.08[a]	53.32 ± 1.97[a]
Protein (%)	8.85 ± 0.32[a]	8.49 ± 0.36[a]	9.01 ± 0.25[a]
Uronic acid (%)	29.92 ± 1.25[a]	26.08 ± 1.32[b]	23.21 ± 0.93[c]

Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$) by Bonferroni t-test.

2.5.5. Ferric-reducing antioxidant power (FRAP) assay

The amount of total antioxidant capacity was carried out using a modified method of FRAP described by Benzie and Strain (1996). A standard curve was prepared using different concentrations (100–1000 μmol/L) of $FeSO_4 \cdot 7H_2O$. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 μmol/L $FeSO_4 \cdot 7H_2O$.

2.6. Statistical analysis

All experiments were performed at least in duplicate, and analyses of all samples were run in triplicate and averaged. Statistical analysis involved use of the Statistical Analysis Systems (SAS, version 8.1) software package. The results were presented as means of three determinations ± SD (standard deviation). The results obtained were analyzed using one-way analysis of variance (ANOVA) for mean differences among the samples. p-Values of <0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Yields and chemical compositions of GLP

The selective dehydration of polysaccharides without the protection of cell wall by an appropriate method is very important. Both yield and activity of polysaccharides are strongly dependent on the type of dehydration and drying temperature employed, due to the different potential of compounds with different denaturalization temperature. The yields of three crude GLP samples were 3.37% for GLP-H, 3.29% for GLP-V and 3.41% for GLP-F, respectively. There are not significant difference in the yield of polysaccharides obtained by three drying methods ($p > 0.05$).

Some polysaccharides contain neutral sugar and uronic acid, and they are usually conjugated with other components such as protein to exhibit various activities. So it was necessary to analyse the contents of neutral sugar, uronic acid and protein in three polysaccharides. The neutral sugar contents, protein contents and uronic acid contents of three polysaccharides are given in Table 1. The neutral sugar contents of three polysaccharides were similar and ranged from 53.32% (GLP-H) to 55.65% (GLP-F). There is an insignificant difference ($p > 0.05$) in the neutral sugar content among three polysaccharides. The uronic acid contents of three polysaccharides followed the order: GLP-F > GLP-V > GLP-H and were 29.92%, 26.08% and 23.21%, respectively. The significant difference in the uronic acid contents may be related to the difference among the oxygen concentration of three drying methods. Protein contents of three polysaccharides varied from 8.49% to 9.01%. GLP-H had the highest protein content (9.01%) followed by GLP-F (8.85%) and GLP-V (8.49%).

3.2. Scavenging effect on hydroxyl radical

Hydroxyl radical scavenging mechanism was related to the transition metal ions. In the absence of transition metal ions, hydrogen peroxide was fairy stable. However, hydroxyl radicals acted in superoxidation by hydrogen peroxide with metal ions, usually ferrous or cupper. The molecules that could chelate iron and render them inactive in Fenton reaction might have scavenging ability on hydroxyl radical (Macdonald, Galley, & Webster, 2003). Hydroxyl radicals were generated by reaction of iron–EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products upon heating with 2-thiobarbituaric acid under acid conditions, yield a pink tint. Added hydroxyl radical scavengers compete with deoxyribose for the resulted hydroxyl radicals and diminish tint formation (Cheng, Ren, Li, Chang, & Chen, 2002). The above-mentioned model was used to evaluate the hydroxyl radicals scavenging ability of three polysaccharides.

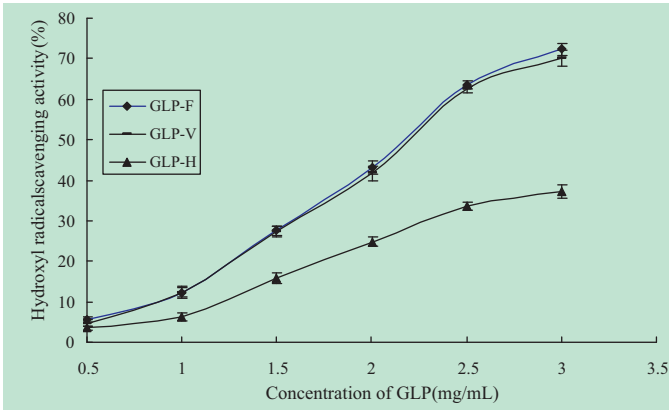

Fig. 1. Scavenging effects of three polysaccharides on hydroxyl radical.

The scavenging effects of three polysaccharides on hydroxyl radicals are shown in Fig. 1. Hydroxyl radical-scavenging activity of GLP-F and GLP-V exhibited a relatively high level at all tested concentrations. The scavenging effects of three polysaccharides on the hydroxyl radical followed the order: GLP-F > GLP-V > GLP-H and were 63.6%, 62.5%, and 33.6% at the concentration of 2.5 mg/mL, respectively. No significant differences ($p > 0.05$) were found between GLP-F and GLP-V. It was important to note that the hydroxyl radical scavenging ability of GLP-H was always less than 50% when the concentration is from 0.5 to 3 mg/mL, indicating that it had little antioxidant activity. These results showed that GLP-F and GLP-V exhibited a noticeable ability of scavenging hydroxyl radicals. GLP-F and GLP-V had the stronger scavenging ability and the higher content of uronic acid than GLP-H. The outstanding antioxidant activities of GLP-F and GLP-V may be attributed to the higher content of carboxy groups which can reduce the generation of hydroxyl radicals by chelating ferrous ion. The production of $^{\bullet}OH$ is dependent on the content of Fe^{2+} and H_2O_2 according to the Fenton reaction. The hydroxyl radical scavenging abilities were influenced by the different drying method, which caused the changes of uronic acid content of GLP.

Among the reactive oxygen species, hydroxyl radical is considered to be a highly potent oxidant which can react with most biomacromolecules functioning in living cells and induce severe damage to the adjacent biomolecules. Thus, removing hydroxyl radical is important for antioxidant defense in cell or food systems. Therefore, hydroxyl radical scavenging is extremely important to antioxidant work. Our data on the activities of scavenging hydroxyl radical of GLP-V and GLP-F suggested that it was likely to contribute towards the observed antioxidant effect.

3.3. Scavenging effect on superoxide radical

The superoxide radical is a highly toxic species that could be generated by numerous biological and photochemical reactions.

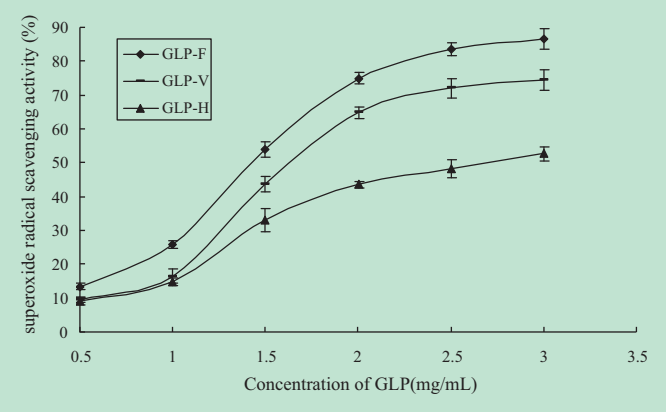

Fig. 2. Scavenging effects of three polysaccharides on superoxide anion.

Superoxide radical can be generated by photochemically reduced flavins and it can reduce nitroblue tetrazolium to blue formazan, measured as a rise in absorbance at 560 nm, which can represent the content of superoxide radicals.

The scavenging effects of three polysaccharides on superoxide radical are shown in Fig. 2. The results indicated that the radical scavenging activity of three polysaccharides followed a concentration-dependent pattern at all tested concentrations. The scavenging activities of three polysaccharides on superoxide anion decreased in order of GLP-F > GLP-V > GLP-H. In the relative lower concentration range of 0.5–1.0 mg/mL, GLP-H exhibited high superoxide radical scavenging activity, which was close to that of GLP-V. However, scavenging activity of GLP-H increased slowly when its concentration was above 1.5 mg/mL. In the higher concentration range of 2.0–3.0 mg/mL, the radical scavenging activity of GLP-H was lower than that of GLP-V. Values of scavenging on superoxide anion of GLP-F, GLP-V and GLP-H were 75.0%, 64.9% and 43.5% at the concentration of 2 mg/mL, respectively. Xing et al. (2005) reported that scavenging activity of vitamin C for superoxide radical was 68.2% at 2.0 mg/mL. Compared to this result, GLP-F had stronger scavenging activity for superoxide radical than vitamin C. The superoxide radical scavenging activities of three polysaccharides were positively correlated with their uronic acid contents. This result was in accordance with the findings of (Li, Liu, Fan, Ai, & Shan, 2011), who found the polysaccharide with higher uronic acid content exhibited stronger scavenging radical activity. In addition, other factors including monosaccharide constituent, molecular weight, and protein content may affect the chelating properties of polysaccharides, and also influence their antioxidant activities. Tsiapali et al. (2001) stated that the free radical-scavenging activity was partially related to monosaccharide constituent. Higher antioxidant activities from *Bryopsis plumosa* were found when the molecular weight increased (Song, Zhang, Zhang, & Wang, 2010). In contrast, a relatively low molecular weight and high protein content appeared to increase the antioxidant activity of polysaccharide from green tea (Chen, Zhang, Qu, & Xie, 2008).

Although superoxide is a relatively weak oxidant, it may decompose to form stronger reactive oxygen species, such as singlet oxygen and hydroxyl radical, which initiate cellular damage, lipid peroxidation and pathological incidents such as arthritis and Alzheimer's disease (Yuan et al., 2005). Superoxide anion is also known to initiate indirectly the lipid peroxidation as a result of the formation of H_2O_2, creating precursors of hydroxyl radical (Meyer & Isaksen, 1995).

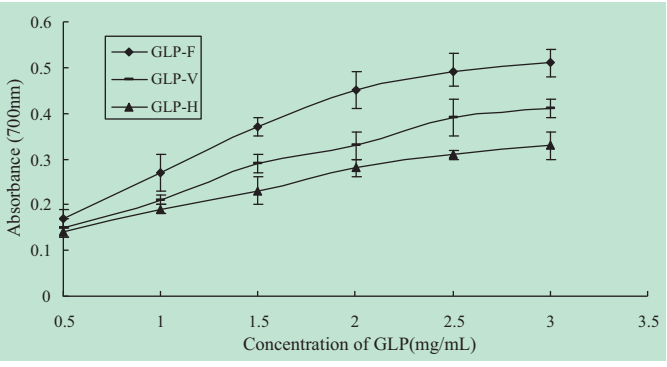

Fig. 3. Reducing power of three polysaccharides.

3.4. Reducing power

It has been reported that there was a direct correlation between antioxidant activity and reducing capacity (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). The reducing properties are generally associated with the presence of reductones, which could donate a hydrogen atom and exert antioxidant action by breaking the free radical chain (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The antioxidant activity was concomitant with the reducing power (Duh, Du, & Yen, 1999; Tanaka, Kuie, Nagashima, & Taguchi, 1998). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In order to elucidate the relationship between the antioxidant activity and the reducing power of three polysaccharides, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of GLP. The reducing powers of all samples are shown in Fig. 3. Among three polysaccharides, reducing power was found to decrease in order of GLP-F, GLP-V and GLP-H, the reducing powers of GLP correlated well with their scavenging ability, indicating that their reducing power contributes to their scavenging ability. The results reported in the present study are in agreement with the result previously reported by Yen and Duh (1993). They found that reducing power is associated with the antioxidant activity. Our data on reducing power of three polysaccharides showed that the polysaccharides can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

3.5. Scavenging effect on DPPH

The DPPH free radical is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Hu, Lu, Huang, & Ming, 2004). Alcoholic solutions of DPPH have a characteristic absorption maximum at 517 nm. The method of scavenging DPPH is based on the reduction of DPPH ethanol solution in the presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form DPPH-H by the reaction (Li, Zhou, & Li, 2007). It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Sanchez-Moreno, 2002). On the basis of this principle, the scavenging effects of GLP-F, GLP-V and GLP-H on the DPPH radical are measured and shown in Fig. 4. The scavenging abilities of three polysaccharides were in a concentration-dependent fashion. At 1.5 mg/mL, the scavenging effect increased to 56.2%, 63.7% and 47.3% for GLP-F, GLP-V and GLP-H, respectively. The results mentioned above implied that GLP might act as electron or hydrogen donator to scavenge DPPH. Interestingly, GLP-V exhibited higher radical scavenging activity than GLP-F at the lower doses (0.5–1.5 mg/mL), whereas the radical scavenging activity of GLP-V was lower than that of GLP-F at the higher doses (2.0–3.0 mg/mL).

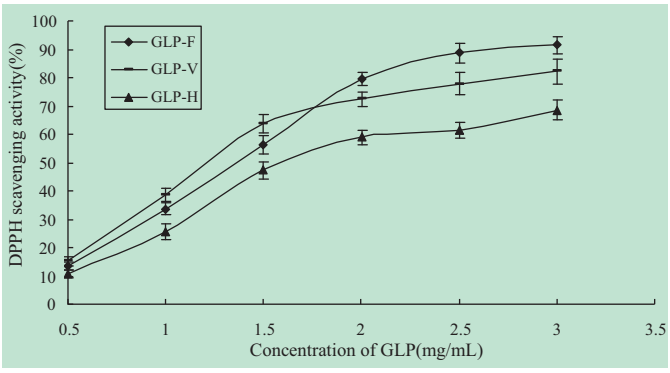

Fig. 4. Scavenging effects of three polysaccharides on the DPPH.

This may be related to its impurity which contain some other phytochemicals, such as triterpenoids and pigments, which also contributed to the total antioxidant activity.

3.6. Ferric-reducing antioxidant power

The FRAP assay treats the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction, and the value reflects the reducing power of antioxidants. The procedure is relatively simple and easy to standardise. This assay is also commonly used for the routine analysis of single antioxidant and total antioxidant activity of plant extracts (W. T. Xu et al., 2009). The antioxidant potentials of different samples were estimated by their ability to reduce the TPTZ–Fe(III) complex to the TPTZ–Fe(II) complex with an maximum absorption at 593 nm. The reduction of absorbance is proportional to the antioxidant content (Benzie & Strain, 1996).

The antioxidant capacities of GLP-F, GLP-V and GLP-H are shown in Fig. 5. The antioxidant capacities of three polysaccharides correlated well with their increasing concentration. The antioxidant capacity of GLP-F was significantly higher than those of GLP-V and GLP-H. At the concentration of 0.2 mg/mL, the FRAP values of GLP-F, GLP-V and GLP-H, were 587, 493 and 471 μmol/L, respectively. These results clearly demonstrated that three polysaccharides possessed antioxidant capacities, especially GLP-F showed strong antioxidant capacity. Interestingly, GLP-H and GLP-V showed an almost identical pattern in FRAP system ($p > 0.05$). However, GLP-V showed the stronger radical-scavenging activities than GLP-H. This could be explained that radical-scavenging activity is only one of several antioxidant mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Zou et al., 2008). The antioxidant mechanism of three polysaccharides obtained from the fruiting bodies of *G. lucidum* by different drying methods is still not fully understood. Therefore, it is suggested that further work could be performed on the possible antioxidant mechanism of three polysaccharides obtained from *G. lucidum* by different drying methods.

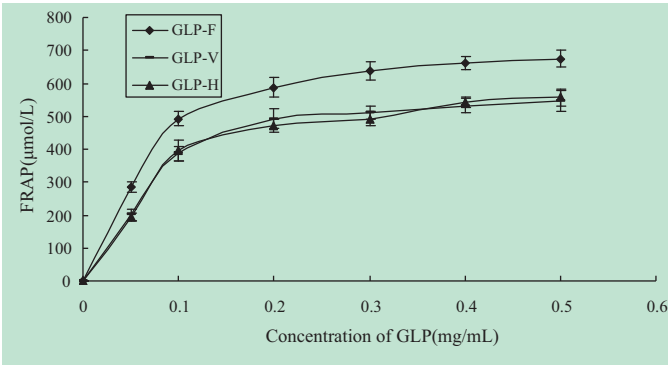

Fig. 5. Ferric-reducing antioxidant power (FRAP) of three polysaccharides.

4. Conclusion

The results of the present work indicate that three polysaccharides obtained from the fruiting bodies of *G. lucidum* by different drying methods had different levels of scavenging effects on free radicals, reducing power and FRAP. The results showed that three polysaccharides exhibited antioxidant activities in a concentration-dependent manner. Among three polysaccharides, GLP-F had the highest scavenging effects on hydroxyl radicals, superoxide radicals, DPPH free radical, and had potential reducing power and ferric-reducing antioxidant power. The results suggested that FD was an appropriate and effective treatment for obtaining the polysaccharide from *G. lucidum*. Antioxidant activities studies indicated GLP-F and GLP-V exhibit greater capacity in scavenging free radicals, which may be related to the high content of uronic acid, which could scavenge free radical by reacting with it. Besides, the antioxidant activity of polysaccharide is usually influenced by various factors combined rather than one single factor. Therefore, further research is needed to elucidate mechanism of antioxidant activity.

Acknowledgements

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